AMENDMENTS TO THE CLAIMS:

The listing of claims will replace all prior versions, and listings, of claims in the application:

Claim 1 (currently amended): A method of domain specific gene evolution of a target nucleic acid encoding ~~an amino acid~~ a polypeptide sequence of interest, said method comprising:

contacting ~~a~~ the target nucleic acid with a recombinase and a first plurality of pairs of single-stranded-targeting polynucleotides which are substantially complementary to each other, wherein each said targeting polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a first predetermined sequence of said target nucleic acid encoding a first domain of a polypeptide, said first plurality of pairs comprising a first library of nucleic acids having mismatches between said targeting polynucleotides and said first predetermined sequence, to form a first library of altered target nucleic acids;

and repeating said contacting on said library of altered nucleic acids whereby said first predetermined sequence undergoes domain specific gene evolution.

Claim 2 (currently amended): A method according to claim 1, further comprising:

contacting said target nucleic acid or said first library of altered target nucleic acids with a second plurality of pairs of single-stranded targeting polynucleotides which are substantially complementary to each other and are not substantially complementary to said first plurality of polynucleotides, wherein each said targeting polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a second predetermined sequence of said target nucleic acid encoding a second domain of said polypeptide, said second plurality of pairs comprising a second library of nucleic acids having mismatches between said second targeting polynucleotides and said second predetermined sequence, to form a second library of altered target nucleic acids whereby said second predetermined sequence undergoes domain specific gene evolution.

Claim 3 (currently amended): A method of domain specific gene evolution comprising:

a) contacting a target nucleic acid encoding a polypeptide of interest with a recombinase and a first pair of single stranded-targeting polynucleotides which are substantially complementary to each other, wherein each said targeting polynucleotide comprises a

homology clamp that substantially corresponds to or is substantially complementary to a first predetermined sequence of said nucleic acid encoding a first domain of said polypeptide to form a first recombination intermediate;
b) contacting said recombination intermediate with a single strand-specific nuclease to form a nicked ~~or open ended~~ target nucleic acid; and
c) reassembling and recombining said nicked or ~~open ended~~ target nucleic acid to evolve a first library of altered target nucleic acids whereby said first predetermined sequence undergoes domain specific gene evolution.

Claim 4 (currently amended): A method according to claim 3 further comprising:

d) ~~combining~~ contacting said target nucleic acid or said first library of altered target nucleic acids with a second pair of single-stranded targeting polynucleotides which are substantially complementary to each other and are not substantially complementary to said first pair of polynucleotides, wherein each targeting polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a second predetermined sequence of said target nucleic acid encoding a second domain of said polypeptide, to form a second recombination intermediate, wherein said contacting of step b) is of said second recombination intermediate with said nuclease.

Claim 5 (cancelled)

Claim 6 (cancelled)

Claim 7 (previously amended): A method of generating a library of altered nucleic acids of a pre-selected target nucleic acid in a chromosomal sequence, said method comprising:
a) contacting a chromosomal nucleic acid comprising a target nucleic acid with at least one recombinase and a first plurality of pairs of single-stranded targeting polynucleotides which are substantially complementary to each other, wherein each said polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a first preselected sequence of said target nucleic acid, said plurality of pairs comprising a first library of nucleic acids having mismatches between said targeting polynucleotides and said first preselected sequence, to form a first library of altered target nucleic acids; and
b) repeating step a) on said library of altered target nucleic acids.

Claim 8 (currently amended): A method according to claim 7 further comprising:

c) ~~adding to~~ contacting said chromosomal nucleic acid or said first library of altered target nucleic acids a second plurality of pairs of single-stranded targeting polynucleotides which are substantially complementary to each other and are not substantially complementary to said first plurality of polynucleotides, wherein each said polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a second preselected sequence of said target nucleic acid, said second plurality of pairs comprising a second library of nucleic acids having mismatches between said targeting polynucleotides and said second preselected sequence, to evolve a second library of altered target nucleic acids, wherein said repeating is on said second library of altered target nucleic acids.

Claim 9 (cancelled)

Claim 10 (currently amended): A method according to any one of claims 1, 2, 3, 4, ~~5, 6,~~ 7, 8, 25, 26, ~~27,~~ and 28 further comprising introducing the resultant product ~~said library of altered target nucleic acids~~ into cells to form a cellular library comprising variant nucleic acid sequences.

Claim 11 (currently amended): A method according to claim 10 further comprising expressing said cellular library of altered target nucleic acids to generate a library of variant polypeptides.

Claim 12 (previously amended): A method according to claim 10 further comprising selecting a cell comprising an altered target nucleic acid having a desired activity.

Claim 13 (previously amended): A method according to claim 10 further comprising selecting a cell comprising an altered target nucleic acid and having a desired phenotype.

Claim 14 (currently amended): A method according to claim 11 further comprising secreting said cellular library of variant ~~amino acid sequences~~ polypeptides.

Claim 15 (previously amended): A method according to claim 10 wherein said recombinase is removed prior to said introducing.

Claim 16 (previously amended): A method according to claim 29 wherein said cell is eukaryotic.

Claim 17 (previously amended): A method according to claim 29 wherein said cell is procaryotic.

Claim 18 (currently amended): A method according to claim 1, 2, 3, 4, ~~5, 6,~~ 7, 8, 25, 26, ~~27,~~ or 28 wherein said targeting polynucleotides are coated with said recombinase.

Claim 19 (currently amended): A method according to claim 1, 2, 3, 4, ~~5, 6,~~ 7, 8, 25, 26, ~~27,~~ or 28 wherein said recombinase is a species of prokaryotic recombinase.

Claim 20 (currently amended): A method according to claim 1, 2, 3, 4, ~~5, 6,~~ 7, 8, 25, 26, ~~27,~~ or 28 wherein said recombinase is a species of eukaryotic recombinase.

Claim 21 (previously amended): A method according to claim 11, wherein said variant polypeptides comprise a plurality of amino acid substitutions.

Claim 22 (currently amended): A method according to claim 1, 2, 3, 4, ~~5, 6,~~ 7, 8, 25, 26, ~~27,~~ or 28 wherein at least one of said targeting polynucleotides further comprises a chemical substituent.

Claim 23 (currently amended): A method according to claim 1, 2, 3, 4, ~~5, 6,~~ 7, 8, 25, 26, ~~27,~~ or 28 wherein said target ~~amino~~ nucleic acid comprises a ~~complementary~~ complementarity determining region

Claim 24 (currently amended): A method according to claim 1, 2, 3, 4, ~~5, 6, 7, 8,~~ 25, 26, ~~27,~~ or 28 wherein said target nucleic acid comprises an expression vector.

Claim 25 (previously added): A method according to claim 1, further comprising:

contacting all or part of said first library of altered nucleic acids with a second plurality of pairs of single-stranded targeting polynucleotides which are substantially complementary to each other and are not substantially complementary to said first plurality of polynucleotides, wherein each said targeting polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a second predetermined sequence of said target nucleic acid encoding a second domain of said polypeptide, said second plurality of pairs comprising a second library of nucleic acids

having mismatches between said second targeting polynucleotides and said second predetermined sequence, to form a second library of altered target nucleic acids.

Claim 26 (previously added): A method according to claim 3 further comprising:

d) contacting said first recombination intermediate with a second pair of single-stranded targeting polynucleotides which are substantially complementary to each other and are not substantially complementary to said first pair of polynucleotides, wherein each targeting polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a second predetermined sequence of said target nucleic acid encoding a second domain of said polypeptide, to form a second recombination intermediate, wherein said contacting of step b) is of said second recombination intermediate with said nuclease.

Claim 27 (previously added): A method according to claim 5 further comprising:

c) contacting all or part of said first library of altered target nucleic acids with at least one recombinase and a second plurality of pairs of single-stranded targeting polynucleotides which are substantially complementary to each other and are not substantially complementary to said first plurality of polynucleotides, wherein each polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a second preselected sequence of said target nucleic acid, said second plurality of pairs comprising a second library of nucleic acids having mismatches between said targeting polynucleotides and said second preselected sequence, to evolve a second library of altered target nucleic acids,

wherein said repeating is on said second library of altered target nucleic acids.

Claim 28 (previously added): A method according to claim 7 further comprising:

c) contacting all or part of said first library of altered target nucleic acids with at least one recombinase and a second plurality of pairs of single-stranded targeting polynucleotides which are substantially complementary to each other and are not substantially complementary to said first plurality of polynucleotides, wherein each said polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a second preselected sequence of said target nucleic acid, said second plurality of pairs comprising a second library of nucleic acids having mismatches between said targeting polynucleotides and said second preselected sequence, to evolve a second library of altered target nucleic acids,

wherein said repeating is on said second library of altered target nucleic acids.

Claim 29 (currently amended): A method according to claim ~~1, 2,~~ 3, 4, ~~5, 6, 7, 8, 25,~~ or 26~~, 27, or 28~~ further comprising contacting said recombination intermediate with a recombination proficient cell.